# Pre-treatment neutrophil-to-lymphocyte ratio as predictor of adverse outcomes in patients undergoing radical cystectomy for urothelial carcinoma of the bladder

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Abstract: BACKGROUND: An elevated neutrophil-to-lymphocyte ratio (NLR) is associated with poor outcome in various tumours. Its prognostic utility in patients with urothelial carcinoma of the bladder (UCB) undergoing radical cystectomy (RC) is yet to be fully elucidated. METHODS: A cohort of patients undergoing RC for UCB in a tertiary referral centre between 1992 and 2012 was analysed. Neutrophil-to-lymphocyte ratio was computed using complete blood counts performed pre-RC, or before neo-adjuvant chemotherapy where applicable. Time-dependent receiver operating characteristic curves were used to determine the optimal cutoff point for predicting recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS). The predictive ability of NLR was assessed using Kaplan-Meier analyses and multivariable Cox proportional hazards models. The likelihood-ratio test was used to determine whether multivariable models were improved by including NLR. RESULTS: The cohort included 424 patients followed for a median of 58.4 months. An NLR of 3 was determined as the optimal cutoff value. Patients with an NLR 3.0 had significantly worse survival outcomes (5y-RFS: 53% vs 64%, log-rank P=0.013; 5y-CSS: 57% vs 75%, log-rank P<0.001; 5y-OS: 43% vs 64%, log-rank P<0.001). After adjusting for disease-specific predictors, an NLR 3.0 was significantly associated with worse RFS (HR=1.49; 95% CI=1.12-2.0, P=0.007), CSS (HR=1.88; 95% CI=1.39-2.54, P<0.001) and OS (average HR=1.67; 95% CI=1.17-2.39, P=0.005). The likelihood-ratio test confirmed that prognostic models were improved by including NLR. CONCLUSIONS: Neutrophil-to-lymphocyte ratio is an inexpensive prognostic biomarker for patients undergoing RC for UCB. It offers pre-treatment prognostic value in addition to established prognosticators and may be helpful in guiding treatment decisions.

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# Pre-treatment neutrophil-to-lymphocyte ratio as predictor of adverse outcomes in patients undergoing radical cystectomy for urothelial carcinoma of the bladder

**Running Title:** Prognostic role of NLR in bladder cancer

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**Abstract**

**Background:** An elevated neutrophil-to-lymphocyte ratio (NLR) is associated with poor outcome in various tumors. Its prognostic utility in patients with urothelial carcinoma of the bladder (UCB) undergoing radical cystectomy (RC) is yet to be fully elucidated.

**Methods:** A cohort of patients undergoing RC for UCB in a tertiary referral center between 1992 and 2012 was analyzed. NLR was computed using complete blood counts performed pre-RC, or prior to neo-adjuvant chemotherapy where applicable. Time dependent receiver operating characteristic curves were used to determine the optimal cut-point for predicting recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS). The predictive ability of NLR was assessed using Kaplan-Meier analyses and multivariable Cox-proportional hazards models. The likelihood-ratio test was used to determine if multivariable models were improved by including NLR.

**Results:** The cohort included 424 patients followed for a median of 58.4 months. An NLR of 3.0 was determined as the optimal cut-off value. Patients with an NLR ≥3.0 had significantly worse survival outcomes (5y-RFS: 53% vs. 64%, log-rank $P$=0.013; 5y-CSS: 57% vs. 75%, log-rank $P$<0.001; 5y-OS: 43% vs. 64%, log-rank $P$<0.001). After adjusting for disease-specific predictors, an NLR ≥3.0 was significantly associated with worse RFS (HR=1.58; 95%CI=1.23-2.05, $P$<0.001), CSS (HR=1.95; 95%CI=1.43-2.65, $P$<0.001) and OS (average HR=1.65; 95%CI=1.20-2.29, $P$<0.001). The likelihood-ratio test confirmed that prognostic models were improved by including NLR.

**Conclusion:** NLR is an inexpensive prognostic biomarker for patients undergoing RC for UCB. It offers pre-treatment prognostic value in addition to established prognosticators, and may be helpful in guiding treatment decisions.

**Keywords:** urinary bladder neoplasms, neutrophils, lymphocytes, biological markers, prognosis, treatment outcome, cystectomy, inflammation

## Introduction

Radical cystectomy (RC) with pelvic lymph node dissection is the standard treatment for muscle-invasive (MI) urothelial carcinoma of the bladder (UCB), and is recommended for patients with non-muscle-invasive (NMI) UCB with high risk of progression (Clark *et al*, 2013). Despite curative intent, disease recurs in a significant proportion of patients and 5-year survival rates of only 40-60% have consistently been reported (Gakis *et al*, 2013).

More aggressive treatment options, such as early RC in patients with high-risk NMI UCB or RC in combination with neo-adjuvant chemotherapy (NAC) or adjuvant chemotherapy (AC) have been shown to improve outcomes (Leow *et al*, 2013; Meeks *et al*, 2012; Raj *et al*, 2011; Sternberg *et al*, 2013). However, employing aggressive strategies unselectively to all patients carries the risk of overtreatment in patients with favorable prognoses. Improved risk-stratification will individualize the use of such approaches. At this time however, risk stratification based on clinico-pathological data alone is unlikely to be sufficient for optimal treatment decision-making (Canter *et al*, 2011; Ficarra *et al*, 2005; Shariat *et al*, 2007). Thus, novel prognostic markers are needed to improve stratification, and, eventually outcomes, of patients with UCB.

Inflammation plays an important role in the development and progression of many malignancies (Grivennikov *et al*, 2010; Hanahan & Weinberg, 2011). Putative mechanisms include the increased supply of factors that promote carcinogenesis and tumor progression by cells of the innate immune systems (i.e. neutrophils) and decreased anti-tumoral response by immune cells of the adaptive system (i.e. lymphocytes) (Hanahan & Weinberg, 2011). The neutrophil to lymphocyte ratio (NLR), which can easily be calculated from routine complete blood counts (CBCs) with differentials, is an emerging marker of host inflammation and has been shown to be

an independent prognosticator for a variety of solid malignancies (Guthrie *et al*, 2013; Proctor *et al*, 2012; Templeton *et al*, 2013). However, there is sparse data on the prognostic role of NLR in patients with UCB (Demirtas *et al*, 2013; Gondo *et al*, 2012; Krane *et al*, 2013).

The objective of our investigation was to evaluate the association between pre-treatment NLR and survival in patients undergoing RC for UCB in a cohort of patients from a tertiary care center.

## Materials and Methods

*Patients and data sources*

Using our institutional database, patients who underwent RC between January 1, 1992 and December 31, 2012 were retrospectively identified. Patients were excluded if CBCs with differentials were unavailable for analysis (n=14), or if they had a history of conditions that may have influenced blood cell lines (connective tissue disease: n=4, malignant lymphoma: n=3, leukemia: n=2, and Human Immunodeficiency Virus (HIV) infection: n=1). Patients undergoing RC for salvage therapy following failed chemo-radiation (n=20) were excluded due to the potential influence of prior chemotherapy on blood cell lines. Patients with non-urothelial cancers (n=9), or for primary prostatic urothelial carcinoma (n=5) were also excluded in order to maintain a homogenous cohort. Electronic hospital chart review was performed to collect clinical parameters including blood work results. Mortality data was obtained through the Princess Margaret Cancer Centre Cancer Registry. Institutional research-ethics board approval was obtained.

*Primary study exposure*

The date of initiation of treatment for each patient was defined as the date of RC, or date of initiation of NAC for patients who received NAC. All patients were seen for medical assessment prior to the initiation of treatment. Generally, NLR was calculated using neutrophil and lymphocyte counts from a routine CBC with differentials done on the same day as these visits (median of 6 days (interquartile range (IQR): 2-10 days) prior to initiation of treatment). Review of the pre-treatment clinic notes did not reveal any symptoms or signs of infections that may have influenced the NLR.

*Outcomes measures*

Patients were generally seen at six to eight weeks after the RC, and otherwise every three to six months early on for periodic physical exam, imaging to rule out hydronephrosis or tumor recurrence, and urethroscopy if indicated. Follow up subsequently became less intensive based on individual physician's practice patterns and clinical suspicion. The outcome measures were recurrence-free survival (RFS), cancer-specific survival (CSS) and overall survival (OS), measured in months from the date of initial treatment.

*Statistical analysis*

Statistical analyses were performed using SAS v9.3 (SAS Institute Inc, Cary, NC, USA). Clinical characteristics were compared between patients with NLR values above and below the optimal cut-point (see below) using the Wilcoxon rank-sum test for continuous variables and Pearson's Chi-Square test for categorical variables.

In the literature, there is heterogeneity in the NLR cut-points used (Guthrie *et al*, 2013). Therefore, in order to determine the optimal cut-point for clinical use, time-dependent receiver-operating characteristic (ROC) curves were created for each outcome measure at 12, 24, 36, 48, and 60 months (Heagerty & Zheng, 2005; Lu & Liu, 2006). NLR values between 1.5 and 6.0 were considered in 0.5 increments. The optimal NLR value for each outcome at a given time point was identified by minimizing the distance from the ROC curve to the top left corner of the ROC plot (and thus optimizing both sensitivity and specificity) (Perkins & Schisterman, 2006).

Kaplan-Meier analyses with log-rank tests were then used to compare survival outcomes between patients with NLR values above versus below the optimal cut-point. To determine how NLR can

influence risk stratification in the pre-treatment and post-cystectomy settings among patients with localized disease (without evidence of nodal disease), we performed additional Kaplan-Meier analyses stratifying by clinical and pathological stage, respectively. In these analyses, patients receiving NAC or AC were excluded to have a more clear impression of how NLR values impact the natural history of disease.

Univariate and multivariable Cox-proportional hazards models were built for each survival outcome. Multivariable models adjusted for *a priori* defined patient-related risk factors (age, gender, Charlson comorbidity index), tumor-related variables (pathological T-stage), treatment-related parameters (year of RC, use of NAC or AC, and surgical margin status), and hematologic parameters (hemoglobin and platelet counts). AC was operationalized as a time-varying covariate to address survivor treatment bias (Austin *et al*, 2006). A robust sandwich covariance matrix estimator was used to account for clustering of outcomes by surgeon (Lin & Wei, 1989). The additional value to the models provided by NLR was evaluated using the Likelihood-ratio test to compare models for each survival outcome with and without NLR. Statistical model assumptions, including the proportional hazards assumption were tested (Hess, 1995).

In order to ensure that our use of a cut-point did not introduce bias (Royston *et al*, 2006), we performed a sensitivity analysis analyzing NLR as a continuous variable with log-transformation (due to its skewed distribution). Given that other studies have not included patients receiving NAC (Gondo *et al*, 2012), we also performed a sensitivity analysis excluding such patients. All tests were two-sided with *P*-values $<0.05$ considered statistically significant.

**Results**

The final study cohort consisted of 424 patients with a median follow up of 58.4 months (IQR: 21.3-94.5 months). The cohort characteristics are described in Table 1. Overall, 138 patients (32.6%) had cancer recurrence and 178 (42.0%) died, of which 110 (25.9%) died of UCB.

In time-dependent ROC curve analyses, an NLR cut-point of 3.0 minimized the distance from the ROC curve to the top-left of the plot for 14 out of 15 time points across the three outcome measures (Supplement Table 1). Given that this cut-point was among those used by other studies (Guthrie *et al*, 2013), we proceeded to use this as the optimal cut-point in our study.

There were 216 (50.9%) patients who had an NLR value ≥3.0 (Table 1). These patients had significantly lower hemoglobin values, higher platelet counts, and a higher Charlson co-morbidity index, and were less likely to receive AC. They were more likely to have pT3-4 disease (53.9% vs. 38.0%), but there was no significant difference in pN-stage.

In univariate Kaplan-Meier analyses NLR ≥3.0 versus <3.0 was associated with increased probability of recurrence (5y-RFS: 53% vs. 64%, log-rank $P$=0.013, Figure 1a), cancer-specific mortality (5y-CSS: 57% vs. 75%, log-rank $P$<0.001, Figure 1b) and overall mortality (5y-OS: 43% vs. 64%, log-rank $P$<0.001; Figure 1c).

In univariate Cox models, an NLR ≥3.0 was associated with increased risk of recurrence (HR=1.53, 95%CI=1.23-1.89, $P$<0.001, Table 2), cancer-specific mortality (HR=1.88, 95%CI=1.52-2.33, $P$<0.001, Table 3) and overall mortality (HR=1.80, 95%CI=1.48-2.20, $P$<0.001, Table 4).

Upon adjusting for confounders using multivariable models, NLR remained significantly associated with increased risk of recurrence (HR=1.58, 95%CI=1.23-2.05, $P$<0.001, Table 2), cancer-specific mortality (hazard ratio (HR)=1.95, 95%CI=1.3-2.65, $P$<0.001, Table 3) and overall mortality (average HR=1.65; 95%CI=1.20-2.29, $P$<0.001, Table 4). Of note, the proportional hazards assumption was satisfied for the models for RFS and CSS, but not for OS. This would suggest that the HR for the association between NLR and OS is not constant but varies as a function of time. Therefore the HR presented in Table 4 represents an average value across the study period. As others have presented such findings(Lipscombe *et al*, 2013), Figure 2 shows how the HR changes as a function of time from initial treatment (with the corresponding model that includes an NLR*time interaction term shown in Supplementary Table 2). NLR has the strongest association with increased risk of overall mortality early on, and then gradually decreases. This association remained statistically significant up to approximately 50 months from start of treatment.

Associations between NLR and increased risk of adverse survival outcomes remained statistically significant when NLR was analyzed as a log-transformed continuous variable, as well as in the sensitivity analysis excluding patients who received NAC (data not shown). Using the Likelihood-ratio test and comparing multivariable Cox-models with and without NLR, it was determined that NLR significantly improved models for RFS ($P$=0.013), CSS ($P$=0.001), and OS ($P$=0.003).

Lastly, we performed exploratory analyses to assess the potential prognostic impact of using NLR when risk-stratifying patients into two scenarios. For these analyses, patients receiving NAC or AC were excluded, to better reflect the natural history of disease and avoid confounding

from adjunctive treatment. The first scenario assessed patients without *clinical* evidence of nodal disease (cN0), where risk stratification may guide initial management in the pre-treatment setting. We sought to determine if NLR further stratified patients beyond their clinical stage, analyzing its impact in clinically NMI UCB versus MI UCB disease subgroups. In these Kaplan-Meier analyses (Figure 3a-c), NLR added valuable prognostic information. Patients with clinical NMI UCB appeared to separate into two groups, with those with clinical NMI UCB and NLR ≥3.0 manifesting survival outcomes comparable to clinical MI UCB. The second scenario was the post-operative setting among patients who were pN0 (where a decision must be made regarding the use of AC). NLR further stratified patients within pT-stage categories (Figure 4a-c). Notably among patients with organ-confined (pT0-pT2) disease, NLR identified a subset of patients who were at increased risk of adverse oncologic outcomes.

## Discussion

The host inflammatory response has gained increasing attention in oncology research. Infiltrating cells of the immune system are constituents of virtually all neoplasms (Hanahan & Weinberg, 2011). While initially thought to represent an anti-tumoral response, immune cells, particularly those of the innate immune system, also exhibit effects that promote carcinogenesis and cancer progression (Grivennikov *et al*, 2010; Hanahan & Weinberg, 2011). Proposed mechanisms include increased supply of growth factors, survival factors, pro-angiogenic factors, extracellular matrix-modifying enzymes (which can facilitate invasion and metastasis), and inductive signals that may lead to epithelial-to-mesenchymal transition (Hanahan & Weinberg, 2011). Thus, there is a biological rationale for using NLR, the ratio of circulating neutrophils (immune cells of the innate system) to lymphocytes (immune cells of the adaptive system), as a measure of the systemic host response when evaluating the association between inflammation and cancer outcomes.

The prognostic role of NLR has been evaluated in numerous epidemiologic studies of various cancers. Higher NLR has been found to be consistently associated with more advanced stage and more aggressive tumor behavior (Guthrie *et al*, 2013; Templeton *et al*, 2013). However, data regarding the association of NLR and prognosis for UCB after RC is still scarce. To date, only three small studies have been published in this population (Demirtas *et al*, 2013; Gondo *et al*, 2012; Krane *et al*, 2013). Gondo and colleagues were the first to describe an association between higher NLR (> 2.5) and CSS in a cohort of 189 patients undergoing RC (Gondo *et al*, 2012). Demirtas and colleagues (201 patients) reported no association between NLR (>2.5) and OS (Demirtas *et al*, 2013), whereas Krane and colleagues (68 patients) found that an elevated NLR (>2.5) was an independent predictor of extravesical disease and worse OS (Krane *et al*, 2013). In

the latter study however, ten patients received NAC and it is unclear how their calculated NLR based on immediate preoperative bloodwork may have been affected.

To the best of our knowledge, our study has the largest sample size investigating the independent prognostic ability of NLR in patients undergoing RC for UCB. It is the first of its kind to show that pre-treatment NLR is an independent prognostic factor for RFS, CSS and OS. Among patients receiving NAC, we used CBCs collected prior to initiation of chemotherapy to eliminate this potential confounder. In addition, a sensitivity analysis excluding patients receiving NAC was performed to confirm robustness of the findings.

There is heterogeneity in reported thresholds used to define an elevated NLR in the literature (range 2.0 - 7.7) (Templeton *et al*, 2013). This may reflect variations in the host response for different disease sites and stages, or may reflect the different approaches used when determining cut-off values. Not all studies used an accepted method for cut-point determination, and in some instances the rationale for the cut-point decision was not described (Templeton *et al*, 2013). All three previously mentioned studies in the RC population used an optimal NLR cut-point of 2.5 (Demirtas *et al*, 2013; Gondo *et al*, 2012; Krane *et al*, 2013). Gondo and colleagues used the cut-point that generated the lowest p-value in Kaplan-Meier analyses (Gondo *et al*, 2012). It is unclear however, if this cut-point was associated with optimal sensitivity and specificity for adverse oncologic outcomes in their study population. One of the other studies chose 2.5 as their cut-point for consistency with Gondo and colleagues (Krane *et al*, 2013), while the third study did not elaborate on the rationale for their cut-point value (Demirtas *et al*, 2013).

We used time-dependent ROC curves to determine the optimal cut-point for NLR. While ROC curves are conventionally used for binary outcomes to identify points of optimal sensitivity and

specificity, this approach was adapted for survival analyses (Heagerty & Zheng, 2005; Lu & Liu, 2006). In our study, 3.0 was determined optimal cut-point. We felt it was important to identify an *a priori* optimal cut-point both for practical purposes, and to minimize bias. Even so, there is likely a continuous association between NLR and risk of adverse oncologic outcomes. This warranted a sensitivity analysis using NLR as a log-transformed continuous variable to ensure we did not introduce any cut-point bias (Royston *et al*, 2006).

Lastly, our exploratory analyses indicate that NLR may better risk-stratify patients in the pre- and post-operative settings in order to guide treatment strategies. In patients with clinically NMI UCB, where there is a high risk of under-staging, and a high risk of disease progression to MI UCB (Chamie *et al*, 2013; Shariat *et al*, 2007; Thomas *et al*, 2012). The NLR may be helpful to identify patients most likely to benefit from early RC. Similarly, NLR may improve post-operative risk stratification to guide use of adjuvant chemotherapy. However, this was not the primary objective of this study, and further work is needed to identify the clinical scenarios in which NLR may be helpful.

There are limitations to our study. Firstly, this is a retrospective, single-institution observational study. Secondly, our study included patients across a long recruitment period, during which practice patterns might have changed. We addressed this by including year of cystectomy in the multivariable model. Thirdly, we did not measure NLR after RC and therefore cannot investigate if post-RC improvement of NLR has a predictive value. Finally, we are unable to determine whether the outcomes following NAC or AC are different among those patients with high vs. low NLR due to the limited number of patients receiving NAC or AC in our cohort.

In conclusion, NLR is an inexpensive hematologic test based on commonly measured parameters that predicts RFS, CSS and OS in patients with UCB undergoing RC, independent of well-established patient-related and tumor-related predictors. While our results suggest that NLR may have a role as a prognostic biomarker in the pre-RC and post-RC settings, further studies are needed to maximize the clinical utility of NLR.

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**Titles and legends to figures**

**Figure 1 (a-c):** Kaplan-Meier curves for recurrence-free survival (a), cancer specific survival (b), and overall survival (c) for patients with a neutrophil-to-lymphocyte ratio (NLR) <3 and ≥ 3.

**Figure 2:** Instantaneous hazard ratio versus time with corresponding confidence intervals for OS LCL = Lower 95% confidence limit, UCL = Upper 95% confidence limit.

**Figure 3 (a-c):** Kaplan-Meier curves for recurrence-free survival (a), cancer specific survival (b), and overall survival (c) for patients without *clinical* evidence of nodal disease (cN0) and clinically non-muscle invasive bladder cancer (NMIBC) or muscle invasive bladder cancer (MIBC) and a neutrophil-to-lymphocyte ratio (NLR) <3 or ≥ 3, respectively.

**Figure 4 (a-c):** Kaplan-Meier curves for recurrence-free survival (a), cancer specific survival (b), and overall survival (c) for patients with organ-confined bladder cancer (pT0-2 pN0) or non organ-confined bladder cancer (pT3-4 pN0) and a neutrophil-to-lymphocyte ratio (NLR) <3 or ≥ 3, respectively.